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# The Effect Of Diet On The Glucose Content Of Chicken Blood

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# A C K N O W L E D G M E N T

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# I N T R O D U C T I O N

The origin of the glucose found in the blood of animals may be traced to several sources. When a carbohyirate diet is ingested the complex carbohydrates of the diet are converted into monosaccharides or simple sugars. These sugars are generally glucose, fructose, and galactose. The last two sugars, however, are transformed in part into glucose. Fructose, when fed in large amounts, is rapidly changed into glycogen. Galactose is not stored as readily, and if given in excessive doses is partly excreted in the urine. It is interesting to note, however, that the sugar which is lost in this way is not altogether galactose, but some other sugars which appear to be isomeric with it. Deuel and Chambers(1) express the view that both fructose and galactose may first be broken down into trioses and then synthesized into glucose. They have been able to show in phlorhizinized dogs a quantitative conversion of fructose into glucose. The conversion of galactose to glucose is not quantitative, as shown by the fact that Deuel and Chambers were able to recover only about 88 per cent of the theoretical amount in the urine of these animals.

The carbohydrates of the diet do not constitute the only source of glucose, for glycerol resulting from fat digestion may be converted into glucose. Chambers and Deuel[2] have recently shown a practically complete conversion of glycerol to glucose in a number of the phlorhizinized dogs with which they have worked.

Another possible source of glucose is found in the proteins of the diet. Certain amino acids resulting from protein digestion are readily converted into sugar and glycogen during metabolism. Investigations have shown that the sugar-forming amino acids are glycine, alanine, aspartic acid, arginine, glutamic acid, and proline. The conversion of glycine into sugar in the animal body has been definitely established, while Ringer and Lusk[3] have shown the complete conversion of alanine into glucose in phlorhizin diabetes. These two authors have also shown that in phlorhizinized dogs the equivalent of three carbon atoms of the four in aspartic acid can be accounted for in the form of extra glucose in the urine. Lusk[4] has shown that three of the five carbon atoms in glutamic acid are converted into glucose in the completely diabetic animal. Further investigations have shown that in 100 grams of protein there is a sufficient amount of sugar-forming amino acids to yield about 58 grams of glucose.

While the sugar from these sources passes into the circulation the average glucose content of normal blood is somewhat less than 0.1 per cent, because most of the sugar is taken to the liver and stored as glycogen.

A large number of investigations have been made to show how diet and various substances, temperature, and muscular activity influence the blood sugar level of animals in health and in disease. A carbohydrate diet usually increases the glucose content of blood. The increase is noted more quickly after the ingestion of monosaccharides than after the ingestion of the more complex carbohydrates. After the ingestion of 100 grams of glucose the increase in the sugar of the blood sometimes occurs in three minutes.

Generally speaking, the factors which influence the sugar or glucose content of the blood of animals may be classified as physical and metabolic. In disease the physical factors would include those cases of retention due to alteration or destruction of permeable membranes in the excretory organs, such as the kidneys and liver. The accumulation of nitrogenous waste products in certain forms of nephritis, or the hypercholesterolemia associated with obstruction of the biliary ducts by gall stones, are examples of retention brought about by such processes. In the so-called metabolic diseases alteration in the chemistry and glucose content of the blood may be induced by increased or diminished formation or utilization of the various constituents. Thus the deficient utilization of carbohydrates and the resultant increase in the sugar of the blood are the outstanding features of diabetes mellitus. Without multiplying examples, suffice it to say that changes in the blood sugar level need not be anticipated unless some condition effecting formation, utilization or elimination is suspected.

It is generally known that a carbohydrate diet will increase the sugar content of the blood, while a high fat diet lowers the blood sugar level. There are other factors, however, which may influence the blood sugar level. Fuss[5] has made a study of blood sugar levels in ether - narcotized dogs. He reported that the analysis of blood from various vessels of the body showed that there is no loss of sugar from the periphery, nor any change in the ability of the tissues to take up sugar. The hyperglucemia which he observed was considered as due to loss of liver glycogen. Salishcheu[6] has shown that nicotine and tobacco smoke influence the blood sugar level. He states that doses of 0.5 mg. of nicotine produced a hypoglucemia of 1.5 to 49.6% in rabbits, while doses of 1 mg. gave an initial rise in blood sugar of 7.7 - 51% and later a fall of 2.5 - 10.9%. Tobacco smoke in toxic doses gave a fall in blood sugar of 18.3 - 61.7%. Genaud[7] observed the quantitative variations of some chemical constituents of the blood in the normal and in the nephritic dog as functions of various diets. He reported that the chloride, cholesterol, and uric acid of the blood remain the same in amount in the nephritic as in the normal dog. The nitrogen of the blood in the normal dog was not altered in quantity by the absorption of meat. He stated further, however, that the blood sugar presents important variations in the nephritic animal with change of food. According to the work of McCoy[8] the sugar in the blood of lamprey eels decreases during spawning.

Further observations on the effect of various substances on the blood sugar level in animals have been made by Chopra, Bose, and De.[9] These investigators have studied the effect of opium on blood sugar. They assert that opium in 1--6 grain doses may reduce blood sugar in early and mild diabetes, while in severe cases the blood sugar is actually increased. Opium increases the renal threshold for sugar in some subjects, so that, while urinary sugar disappears, blood sugar increases. In normal subjects, small to moderate doses have no effect on blood sugar. This is also the case in patients suffering from nephritis. Silvette and Britton[10] have shown that cortico-adrenal extract increases blood glucose as well as liver and muscle glycogen in normal young rats, cats, and rabbits.

The influence of muscle work on blood sugar has been shown by Hiramatsu.[11] He reported that white rats fed on rice show, in the fasting state, 134 mg. % blood sugar. During muscle work no matter what the rats' diet, the blood sugar decreases, but gradually rises once more when the work is stopped. In a paper entitled "Digestion and Absorption in Fowls", Macowan and Magee[12] state that the blood sugar of fowls is not reduced to a steady level by prolonged fasting, but is variable. High protein meals cause the blood sugar to fall slightly, but progressively. Meals rich in starch cause increases in five minutes after ingestion; when they contain coarse starch-poor particles the blood sugar fluctuates violently. With finely ground diets rich in starch, fluctuations were absent. Another observation has been made by Krasnjansky and Dsikowsky[13] who followed the periodic changes in the blood sugar content of roosters in the course of twenty-four hours. They have shown that the blood sugar curve of the rooster, whether fed or fasting, shows wave-like variations in the course of the 24 hour period. After a 45 hour fast the blood sugar is lowered and in a number of experiments it was found to range between 109 and 232 mg. %. A certain definite periodicity can be recognized in the daily variations of the curve: a very large rise toward evening, a fall during the night and a rise in the early morning. There is also an indication of a possible use at midnight, but this has not been definitely established.

# P U R P O S E

## Nutrition and Metabolism in the Chicken

The feeds consumed by chickens are used in repairing waste and in providing materials for growth, reproduction, and the storing of fat. The complex character of the body of the chicken and of the egg it lays suggests the nature of the feeds it needs. The body consists of water, minerals, proteins, carbohydrates, fats, and various other organic substances. The egg consists of the albumin or the white, a secretion of the oviduct glands, and the yolk, which is the real cell. The albumin contains more than 87 per cent water and nearly 11 per cent protein. The yolk is less important in the protein content, but is far more complex than the albumin and contains about 50 per cent water and about 50 per cent solids, which consist of egg oil, protein, carbohydrates, fats, minerals, and other substances. The shell of the egg consists largely of calcium. Since the egg is so constructed and so exactly made of the best materials that nature had to offer, we must see the close relation that a diet would have upon the elements that are already present. To keep the protein content the same, the carbohydrate content the same, and the fat content the same, a well balanced ration that puts these things into the egg must be given in the food of the chicken.

Protein is relatively more important than fats and carbohydrates because it is the most hard to get in sufficient quantities. The egg is relatively rich in protein, but the staple grains do not contain enough making it necessary for it to be obtained in additional quantities from some other sources. The principal sources of animal protein are meat scraps, fish meal, and milk. The principal sources of vegetable proteins are soybean meal, peanut meal, cotton-seed meal, and gluten meal.

The three most important requirements of satisfactory rations have to do with the supply of proteins, vitamins, and minerals. There are other requirements, however, which must be met, and one of these has to do with the fat and carbohydrate requirements. This is important because fats and carbohydrates supply the chickens with material for the formation of fat in the body and for the development of energy. Almost all of the staple grains are relatively rich in carbohydrates and fat, and because they are abundant and easily obtained, there seems to be little concern over the normal requirements for poultry-feeding purposes. The character of the fat in some of the animal feeds may be significant, especially so if an excess of fatty acids is present. Since a portion of the carbohydrates consists of fiber, some attention should be given to the fiber content of feeds because fowls do not digest fibers so efficiently as mammals, and there seems to be some danger in providing chickens with an excess.

Maximum egg production is largely controlled by the breeding of the stock as well as by the kinds of feeds given and the methods of feeding.

That a proper supply of mineral matter is of great importance to chickens is shown by the results obtained when feeding rations freed, so far as possible, from the minerals which are essential. Minerals exist in all the essential and vital organs of the body of not only the chicken, but man also; calcium and phosphorous make up about 90 per cent of the skeleton of the body. There is usually a deficiency in the calcium content of the chicken's food. Growing chicks need calcium phosphate as a supplement to the grain diet.

Birds need more mineral feed in proportion to their total feed requirements than most other classes of animals. This is primarily because of the egg shell, and also because the skeleton of the bird requires considerable proportions of various kinds of minerals to keep it in repair. Mineral feed is supplied in the form of crushed oyster shells, or high grade limestone, which are considered of about equal value as a source of calcium for egg shell formation. Lime and phosphorous, which are very important in feeding for egg production, are two of the minerals in steamed bone meal, and the content of from 45 to 50% of phosphate of lime from bones serves the hen well in building up her skeleton and furnishing feathers as well as making eggs.

# The Effect of Cod Liver Oil on the Calcium Metabolism of Young Chickens

The poultry industry contributes materially to the agricultural income of this country and constitutes a valuable home market for a large amount of wheat, corn, other grains, and by-products of the packing houses, dairies, and fishing industries. The increased per capita consumption of poultry and eggs, coupled with the perfection of artificial incubation and brooding equipment, has revolutionized the poultry industry, placing poultry raising on a mass-production basis. As a result of this rapid stride that the poultry industry has taken, present-day poultry nutrition has become a very complicated and difficult problem. The conservation of green matter through the long winter months for the vitamin source has helped to cause the radical change, and, as a consequence, cod liver oil has been found as a remedy. It was through the finding of the properties of the oil that now the farmer can hatch off his chicks during the winter and fear no loss that could have resulted through the lack of the vitamins. A good grade of tested cod-liver oil will supply the vitamins formerly obtained from green plants and sunshine, but this does not solve all problems of poultry nutrition. To secure satisfactory growth and bone development, attention must be given to the quantity and quality of proteins, supplementary sources of phosphorous and calcium, the ratio of these two minerals, and the influence of vitamins on the metabolism of the various constituents of the ration.

Holmes and Pigott[14] working in the E. L. Patch Research Laboratories in Boston have engaged in a study of these nutrition factors for the last three years, and the results have served as a guide for modifying the usual type of "growing mash". By making a distinct change in the proteins, lowering the calcium content, changing the calcium phosphate ratio and insuring an adequate supply of vitamins, it has been possible to secure more rapid growth and better feathering at a materially lower cost for feed.

Chickens formerly were raised under conditions closely approximating those for wild birds. The hen incubated ten or twelve eggs and brooded the resulting chicks. Ordinarily she was allowed free range, which allowed a wide variety of bugs, works, green plants, and many hours in the sunshine. Under these conditions the owner had no occasion to consider balanced rations, vitamins, or sanitation.

With the advent of artificial incubation, colony brooding, and more recently, the battery brooder system, the problem of poultry nutrition has become extremely complicated. The modern poultryman must operate on an intensive scale and employ expensive equipment, high priced labor, and high priced feed. He must therefore resort to mass production under conditions which little resemble those provided by nature. For instance, chicks are hatched by the thousands during the winter months, when the temperature may be at or below zero.

Since the modern poultryman must operate on such an intensive scale and must brook off his chicks during the winter months when the sunshine and the green plants are less available in sufficient quantity and quality to afford adequate vitamins a supplementary source of vitamins to the poultry ration has become imperative.

The authors stated that this investigation was undertaken to determine the influence of cod-liver oil on calcium metabolism in young chicks when supplementary calcium was obtained from different sources, fed at different levels and was available in different ratios to phosphorous.

In connection, then, with poultry nutrition investigations, of which the foregoing is typical, it seemed desirable to accumulate data concerning the glucose content of chicken blood and the effect of diet on the blood sugar level in the chicken. Since the literature reviewed contained but little information concerning the glucose content of chicken blood, the author wished also to develop a method for the determination of glucose in chicken blood and to test the results of Macowan and Magee.(12)

# EXPERIMENTAL PART

## I. Preparation of Reagents.

(a) Standard Sugar Solution----

2.5 gms. of benzoic acid was dissolved in a liter of boiling water. The solution was then allowed to cool.

1 gm. of pure glucose was then dissolved in 50 cc. of the benzoic acid solution and the mixture transferred to a 100 cc. volumetric flask. It was then diluted to the mark with more of the benzoic acid solution. This was the stock solution from which the standards were made.

(b) 1 cc. if the stock solution was transferred to a 100 cc. volumetric flask, by means of an Ostwald pipette, and filled to the mark and mixed thoroughly. This diluted solution contained 0.1 mg. of glucose per cc. of solution.

(c) Alkaline Copper Tartrate Solution:

40 grams of anhydrous sodium carbonate was dissolved in 400 cc. of water. The clear solution was then mixed with 7.5 grams of tartaric acid, and 4.5 grams of crystalline copper sulphate. The whole was shaken until solution was complete, and then diluted to one liter.

(d) Special Phosphomolybdic Acid Solution:

A mixture consisting of 35 gms. of molybdic acid, 5 gms. of sodium tungstate and 200 cc. of a 10 per cent sodium hydroxide solution was allowed to boil vigorously for 50 minutes. It was then cooled, diluted to 350 cc. and treated with 125 cc. of 85 per cent Phosphoric acid. The mixture was then diluted to 500 cc.

(e) Acid Molybdate Reagent:

600 grams of pure sodium molybdate was transferred to a 2 liter volumetric flask by means of a large funnel and a fine glass rod. Water was added and the mixture shaken until solution was complete. The solution was then diluted to volume and thoroughly mixed. This stock solution was transferred to a 2 liter Florence flask, treated with 0.5 cc. of bromine and

placed aside. 500 cc. of the clear stock solution was transferred to a liter Florence flask and mixed with 255 cc. of 85% phosphoric acid and 150 cc. of cool sulphuric acid (1 volume concentrated sulphuric acid to 3 volumes of water). The bromine was then removed by passing in a current of air for ten minutes. The mixture was then treated with 75 cc. of 99% acetic, mixed and diluted to one liter. When this solution was kept in a cool, dark place away from organic matter it remained colorless for several weeks.

II. Blood Sugar Determination.

The method used in this determination was essentially that of Folin and Wu.(15) For this work a barred Plymouth Rock hen was used. This hen was placed on a fasting diet for 24 hours and then a sample of the blood was taken. The blood was taken by cutting a marginal vein in the wing of the chicken and allowing the blood to drop into a small beaker previously coated with a thin film of sodium oxalate. The sodium oxalate was used to prevent clotting of the blood. When 5 cc. of blood had been drawn, the blood was transferred by means of a Folin and Wu diluting pipette to a 200 cc. Erlenmeyer flask and laked with 7 volumes of distilled water. The laked blood was then treated with 1 volume of phosphomolybdic acid solution and shaken for 5 minutes. The dark brown mixture was then placed in a centrifuge and whirled for ten minutes, whereupon the preciptated proteins separated, leaving the clear blood filtrate. This clear blood filtrate was used in the determination of glucose.

In this determination 2 cc. of the filtrate was treated with 2 cc. of alkaline copper tartrate in a Folin-Wu sugar tube graduated at 25 cc., and in another sugar tube 2 cc. of a standard sugar solution containing 0.5 mg. of glucose was mixed with 2 cc. of the alkaline copper tartrate solution. The tubes were then placed in a boiling water bath and heated for 8 minutes. The tubes were cooled in cold water for 5 minutes without shaking. Each tube was then treated with 4 cc. of acid molybdate solution. After waiting about one minute the mixture was diluted to the mark with water. The contents of each tube were mixed by placing the thumb over the mouth of the tube and rocking back and forth at least 5 times. The tubes were then compared in a Duboscq colorimeter. Calculations were then made according to the following formula:

$$C_1 = \frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times C_2 \times \frac{100}{0.2}$$

where $C_1$ is equal to the number of milligrams of glucose per 100 cc. of blood, and $C_2$, the concentration of the known.

The first determination was run on a sample of blood taken from the hen immediately following a 24-hour period of starvation. The calculations and results are as follows:

$$C_1 = \frac{R_1}{R_2} \times .05 \times \frac{100}{0.2}$$

$$C_1 = \frac{20}{23} \times .05 \times \frac{100}{0.2}$$

$C_1 = 21.5$ mg.

The above value represents the glucose content of chicken blood following the period of starvation.

THE EFFECT OF HIGH CARBOHYDRATE DIET

To show the effect of ingested carbohydrate on the glucose content of chicken blood a diet consisting of the following was used:

33 gms. bread crumbs

20 gms. rice starch

10 gms. lactose

The hen was fed this diet for a period of 3 days with a sufficient amount of water. At the end of the three-day period a sample of the blood was taken and examined for glucose according to the procedure outlined above. The calculations and results were as follows:

$$C_1 = \frac{R_1}{R_2} \times 0.1 \times \frac{100}{0.2}$$

$$C_1 = \frac{15}{25} \times 0.1 \times \frac{100}{0.2}$$

$$C_1 = 30 \text{ mg.}$$

The results here show that the carbohydrate diet produced a decided increase in the glucose content of the blood of the chicken. This is in accord with the work of Macowan and Magee.[12]

The author experienced considerable difficulty in drawing the blood for the above determination because of the rapid rate of clotting of the blood. In view of the accepted mechanism of blood clot formation the author would suggest that a carbohydrate diet and the resulting increase of glucose in the blood may enhance or facilitate the formation of thromboplastin, which is said to figure prominently in the clotting of blood. Further work to test this point is being planned.

## THE EFFECT OF HIGH FAT DIET

The hen was placed on a diet consisting of 33 gms. of bread crumbs and 25 cc. of pure olive oil. The bread crumbs were rolled in the olive oil and fed in the form of a cake. After a day or two of feeding the hen refused to take the diet. It was then necessary to force the ingestion of the meal. This was done by administering the olive oil by mouth from a pipette. She tood the bread alone without any difficulty. After a period of three days a sample of blood was taken and examined by the foregoing method. The following results were obtained:

$$C_1 = \frac{R_1}{R_2} \times .05 \times \frac{100}{0.2}$$

$$C_1 = \frac{20}{30} \times .05 \times \frac{100}{0.2}$$

$$C_1 = 16.66 \text{ mg.}$$

## THE EFFECT OF HIGH PROTEIN DIET

For this determination the hen was placed on a diet consisting of 5 gms. of pure albumin and 33 gms. of bread crumbs. The albumin was dissolved in the smallest quantity of distilled water and administered by mouth as in the case of the olive oil. At the end of a three-day period, during which this diet was fed, a sample of blood was taken and examined as indicated above. The results obtained in this case are as follows:

$$C_1 = \frac{R_1}{R_2} \times .05 \times \frac{100}{0.2}$$

$$C_1 = \frac{10}{26} \times .05 \times \frac{100}{0.2}$$

$$C_1 = 9.5 \text{ mg.}$$

This value is in agreement with the findings of Macowan and Magee.[12]

Further work, however, is being planned to test this point.

## SUMMARY AND CONCLUSION

1. A convenient method for the determination of glucose in chicken blood has been developed.
2. The glucose content of fasting chicken blood is low.
3. A high carbohydrate diet increases the glucose content of chicken blood. The rate of carbohydrate utilization in the chicken was not determined.
4. A high fat diet lowers the glucose content of chicken blood. It is expected, too, that a high fat diet lowers the rate of carbohydrate utilization in the chicken.
5. A high protein diet lowers the blood sugar level of the chicken progressively. This point will be tested in further work.

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